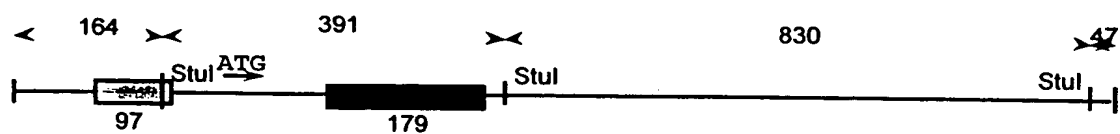
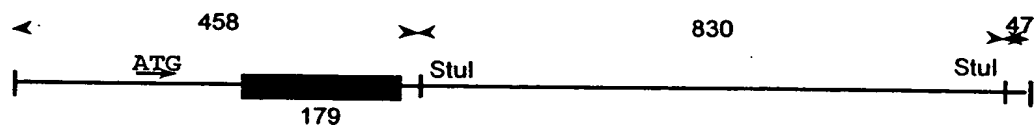


Figure 1

**Full Length:**



**Properly Spliced:**




**Downstream Missplice:**



**Unsplice Intron and Downstream Missplice:**



 Upstream Splice Site (part of the expression vector)

 'Weird' Splice Site (found in the Gcc cDNA sequence)

Figure 2

r rAG/ qua agu Consensus Donor

5' - AAG CCG TTG AGT AGG/ GTA AGC ATC ATG GCT GGC AGC CTC AC 160-

a) unmodified Gcc

Lys Pro Leu Ser Arg Val Ser Ile Met Ala Gly Ser Leu Thr 26
--

5' - AAG CCG TTG AGT AGA GTC TCC ATC ATG GCT GGC AGC CTC AC 160-

\* \* \*\*

b) "modified Gcc"

yyy yyy yyy ync ag/G Consensus Acceptor

5' - TTT CCT GCC CTT GGT ACC TTC AG/C CGC TAT GAG AGT ACA C 340-

a) unmodified Gcc

Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg 83
---

5' TTT CCT GCC CTG GGA ACA TTT TCC CGC TAT GAG AGT ACA C 340-

\* \* \* \* \*

Figure 3 (continued on next page)

b) modified Gcc

Product of Alternatively Spliced mRNA transcript from the  
unmodified Gcc cDNA:

NOTE:

Nucleotides associated with RNA splice-sites are underlined.  
Those believed to be of most importance to splice site-  
recognition are double underlined.

" /" in Consensus Splice Sequence indicates splice site. " \*"  
under a particular nucleotide indicates a base changed in the  
site-directed mutagenesis procedure.

r = C or A, y = c or u, and n = any.

Figure 3 (continued on next page)

Partial produce of the cryptic splicing of Gcc cDNA

5' - AAG CCG TTG AGT AGG CCG CTA TGA GAG TAC AC

Lys Pro Leu Ser Arg<sup>16</sup> Pro Leu STOP<sup>20</sup>!!

Figure 3 (continued)

10	20	30	40	50	60	
123456789012345	678901234567890	123456789012345	678901234567890	123456789012345	678901234567890	
NotI						
NGCGGCCGCTTAGCT	TGACTTAAGAAGGCC	GACGCCATGGAGTTT	TCAAGTCCTTCCAGA			60
		MetGluPhe	SerSerProSerArg			
GAGGAATGTCCCAAG	CCTTTGAGTAGAGTC	TCCATCATGGCTGGC	AGCCTCACAGGTTTG			120
GluGluCysProLys	ProLeuSerArgVal	SerIleMetAlaGly	SerLeuThrGlyLeu			
CTTCTACTTCAGGCA	GTGTCGTGGGCATCA	GGTGCCCCGCCCTGC	ATCCCTAAAAGCTTC			180
LeuLeuLeuGlnAla	ValSerTrpAlaSer	GlyAlaArgProCys	IleProLysSerPhe			
GGCTACAGCTCGGTG	GTGTGTGTCTGCAAT	GCCACATACTGTGAC	TCCTTTGACCCCCCG			240
GlyTyrSerSerVal	ValCysValCysAsn	AlaThrTyrCysAsp	SerPheAspProPro			
ACCTTTCCTGCCCTG	GGAACATTTTCCCGC	TATGAGAGTACACGC	AGTGGGCGACGGATG			300
ThrPheProAlaLeu	GlyThrPheSerArg	TyrGluSerThrArg	SerGlyArgArgMet			
GAGCTGAGTATGGGG	CCCATCCAGGCTAAT	CACACGGGCACAGGC	CTGCTACTGACCCTG			360
GluLeuSerMetGly	ProIleGlnAlaAsn	HisThrGlyThrGly	LeuLeuLeuThrLeu			
CAGCCAGAACAGAAG	TTCCAGAAAGTGAAG	GGATTTGGAGGGGCC	ATGACAGATGCTGCT			420
GlnProGluGlnLys	PheGlnLysValLys	GlyPheGlyGlyAla	MetThrAspAlaAla			
GCTCTCAACATCCTT	GCCCTGTCACCCCCT	GCCCAAATTTGCTA	CTTAAATCGTACTTC			480
AlaLeuAsnIleLeu	AlaLeuSerProPro	AlaGlnAsnLeuLeu	LeuLysSerTyrPhe			
TCTGAAGAAGGAATC	GGATATAACATCATC	CGGGTACCCATGGCC	AGCTGTGACTTCTCC			540
SerGluGluGlyIle	GlyTyrAsnIleIle	ArgValProMetAla	SerCysAspPheSer			
ATCCGCACCTACACC	TATGCAGACACCCCT	GATGATTTCCAGTTG	CACAACTTCAGCCTC			600
IleArgThrTyrThr	TyrAlaAspThrPro	AspAspPheGlnLeu	HisAsnPheSerLeu			
CCAGAGGAAGATACC	AAGCTCAAGATACCC	CTGATTCACCGAGCC	CTGCAGTTGGCCCAG			660
ProGluGluAspThr	LysLeuLysIlePro	LeuIleHisArgAla	LeuGlnLeuAlaGln			
CGTCCCGTTTCACTC	CTTGCCAGCCCCTGG	ACATCACCCACTTGG	CTCAAGACCAATGGA			720
ArgProValSerLeu	LeuAlaSerProTrp	ThrSerProThrTrp	LeuLysThrAsnGly			

Figure 4A(continued on next page)

10	20	30	40	50	60	
123456789012345	678901234567890	123456789012345	678901234567890	123456789012345	678901234567890	
GCGGTGAATGGGAAG	GGGTCACTCAAGGGA	CAGCCCGGAGACATC	TACCACCAGACCTGG			780
AlaValAsnGlyLys	GlySerLeuLysGly	GlnProGlyAspIle	TyrHisGlnThrTrp			
GCCAGATACTTTGTG	AAGTTCCTGGATGCC	TATGCTGAGCACAAG	TTACAGTTCTGGGCA			840
AlaArgTyrPheVal	LysPheLeuAspAla	TyrAlaGluHisLys	LeuGlnPheTrpAla			
GTGACAGCTGAAAAT	GAGCCTTCTGCTGGG	CTGTTGAGTGGATAC	CCCTTCCAGTGCCTG			900
ValThrAlaGluAsn	GluProSerAlaGly	LeuLeuSerGlyTyr	ProPheGlnCysLeu			
GGCTTCACCCCTGAA	CATCAGCGAGACTTA	ATTGCCCGTGACCTA	GGTCCTACCCTCGCC			960
GlyPheThrProGlu	HisGlnArgAspLeu	IleAlaArgAspLeu	GlyProThrLeuAla			
AACAGTACTCACCAC	AATGTCCGCCTACTC	ATGCTGGATGACCAA	CGCTTGCTGCTGCCC			1020
AsnSerThrHisHis	AsnValArgLeuLeu	MetLeuAspAspGln	ArgLeuLeuLeuPro			
CACTGGGCAAAGGTG	GTA CTGACAGACCCA	GAAGCAGCTAAATAT	GTT CATGGCATTGCT			1080
HisTrpAlaLysVal	ValLeuThrAspPro	GluAlaAlaLysTyr	ValHisGlyIleAla			
GTACATTGGTACCTG	GACTTTCTGGCTCCA	GCCAAAGCCACCCTA	GGGGAGACACACCGC			1140
ValHisTrpTyrLeu	AspPheLeuAlaPro	AlaLysAlaThrLeu	GlyGluThrHisArg			
CTGTTCCCCAACACC	ATGCTCTTTGCCTCA	GAGGCCTGTGTGGGC	TCCAAGTTCTGGGAG			1200
LeuPheProAsnThr	MetLeuPheAlaSer	GluAlaCysValGly	SerLysPheTrpGlu			
CAGAGTGTGCGGCTA	GGCTCCTGGGATCGA	GGGATGCAGTACAGC	CACAGCATCATCACG			1260
GlnSerValArgLeu	GlySerTrpAspArg	GlyMetGlnTyrSer	HisSerIleIleThr			
AACCTCCTGTACCAT	GTGGTCGGCTGGACC	GACTGGAACCTTGCC	CTGAACCCCGAAGGA			1320
AsnLeuLeuTyrHis	ValValGlyTrpThr	AspTrpAsnLeuAla	LeuAsnProGluGly			
GGACCCAATTGGGTG	CGTAACTTTGTGCGAC	AGTCCCATCATTTGTA	GACATCACCAAGGAC			1380
GlyProAsnTrpVal	ArgAsnPheValAsp	SerProIleIleVal	AspIleThrLysAsp			
ACGTTTTACAAACAG	CCCATGTTCTACCAC	CTTGCCATTTCAGC	AAGTTCATTCTCTGAG			1440
ThrPheTyrLysGln	ProMetPheTyrHis	LeuGlyHisPheSer	LysPheIleProGlu			

Figure 4A(continued on next page)





10	20	30	40	50	60
123456789012345	678901234567890	123456789012345	678901234567890	123456789012345	678901234567890
NotI					
NGCGGCCGCTTAGCT	TGACTTAAGAAGGCC	GACGCCATGGAGTTT	TCAAGTCCTTCCAGA		60
		MetGluPhe	SerSerProSerArg		
GAGGAATGTCCCAAG	CCTTTGAGTAGAGTC	TCCATCATGGCTGGC	AGCCTCACAGGTTTG		120
GluGluCysProLys	ProLeuSerArgVal	SerIleMetAlaGly	SerLeuThrGlyLeu		
CTTCTACTTCAGGCA	GTGTCGTGGGCATCA	GGTGCCCGCCCCTGC	ATCCCTAAAAGCTTC		180
LeuLeuLeuGlnAla	ValSerTrpAlaSer	GlyAlaArgProCys	IleProLysSerPhe		
GGCTACAGCTCGGTG	GTGTGTGTCTGCAAT	GCCACATACTGTGAC	TCCTTTGACCCCCCG		240
GlyTyrSerSerVal	ValCysValCysAsn	AlaThrTyrCysAsp	SerPheAspProPro		
ACCTTTCCTGCCCTG	GGAACATTTTCCCGC	TATGAGAGTACACGC	AGTGGGCGACGGATG		300
ThrPheProAlaLeu	GlyThrPheSerArg	TyrGluSerThrArg	SerGlyArgArgMet		
GAGCTGAGTATGGGG	CCCATCCAGGCTAAT	CACACGGGCACAGGC	CTGCTACTGACCCTG		360
GluLeuSerMetGly	ProIleGlnAlaAsn	HisThrGlyThrGly	LeuLeuLeuThrLeu		
CAGCCAGAACAGAAG	TTCCAGAAAGTGAAG	GGATTTGGAGGGGCC	ATGACAGATGCTGCT		420
GlnProGluGlnLys	PheGlnLysValLys	GlyPheGlyGlyAla	MetThrAspAlaAla		
GCTCTCAACATCCTT	GCCCTGTCACCCCCT	GCCCAAATTTGCTA	CTTAAATCGTACTTC		480
AlaLeuAsnIleLeu	AlaLeuSerProPro	AlaGlnAsnLeuLeu	LeuLysSerTyrPhe		
TCTGAAGAAGGAATC	GGATATAACATCATC	CGGGTACCCATGGCC	AGCTGTGACTTCTCC		540
SerGluGluGlyIle	GlyTyrAsnIleIle	ArgValProMetAla	SerCysAspPheSer		
ATCCGCACCTACACC	TATGCAGACACCCCT	GATGATTTCCAGTTG	CACAACTTCAGCCTC		600
IleArgThrTyrThr	TyrAlaAspThrPro	AspAspPheGlnLeu	HisAsnPheSerLeu		
CCAGAGGAAGATAACC	AAGCTCAAGATACCC	CTGATTCACCGAGCC	CTGCAGTTGGCCCAG		660
ProGluGluAspThr	LysLeuLysIlePro	LeuIleHisArgAla	LeuGlnLeuAlaGln		
CGTCCCGTTTCACTC	CTTGCCAGCCCCTGG	ACATCACCCACTTGG	CTCAAGACCAATGGA		720
ArgProValSerLeu	LeuAlaSerProTrp	ThrSerProThrTrp	LeuLysThrAsnGly		

Mutations made to the Gcc insert are underlined

Figure 4B (continued on next page)

10	20	30	40	50	60	
123456789012345	678901234567890	123456789012345	678901234567890	123456789012345	678901234567890	
GCGGTGAATGGGAAG	GGGTCACTCAAGGGA	CAGCCCGGAGACATC	TACCACCAGACCTGG			780
AlaValAsnGlyLys	GlySerLeuLysGly	GlnProGlyAspIle	TyrHisGlnThrTrp			
GCCAGATACTTTGTG	AAGTTCCTGGATGCC	TATGCTGAGCACAAG	TTACAGTTCTGGGCA			840
AlaArgTyrPheVal	LysPheLeuAspAla	TyrAlaGluHisLys	LeuGlnPheTrpAla			
GTGACAGCTGAAAAT	GAGCCTTCTGCTGGG	CTGTTGAGTGGATAC	CCCTTCCAGTGCCTG			900
ValThrAlaGluAsn	GluProSerAlaGly	LeuLeuSerGlyTyr	ProPheGlnCysLeu			
GGCTTCACCCCTGAA	CATCAGCGAGACTTA	ATTGCCCGTGACCTA	GGTCCTACCCTCGCC			960
GlyPheThrProGlu	HisGlnArgAspLeu	IleAlaArgAspLeu	GlyProThrLeuAla			
AACAGTACTCACCAC	AATGTCCGCCTACTC	ATGCTGGATGACCAA	CGCTTGCTGCTGCCC			1020
AsnSerThrHisHis	AsnValArgLeuLeu	MetLeuAspAspGln	ArgLeuLeuLeuPro			
CACTGGGCAAAGGTG	GTA <sup>U</sup> CTGACAGACCCA	GAAGCAGCTAAATAT	GTT <sup>U</sup> CATGGCATTGCT			1080
HisTrpAlaLysVal	ValLeuThrAspPro	GluAlaAlaLysTyr	ValHisGlyIleAla			
GTACATTGGTACCTG	GACTTTCTGGCTCCA	GCCAAAGCCACCCTA	GGGGAGACACACCGC			1140
ValHisTrpTyrLeu	AspPheLeuAlaPro	AlaLysAlaThrLeu	GlyGluThrHisArg			
CTGTTCCCCAACACC	ATGCTCTTTGCCTCA	GAGGCCTGTGTGGGC	TCCAAGTTCTGGGAG			1200
LeuPheProAsnThr	MetLeuPheAlaSer	GluAlaCysValGly	SerLysPheTrpGlu			
CAGAGTGTGCGGCTA	GGCTCCTGGGATCGA	GGGATGCAGTACAGC	CACAGCATCATCAGC			1260
GlnSerValArgLeu	GlySerTrpAspArg	GlyMetGlnTyrSer	HisSerIleIleThr			
AACCTCCTGTACCAT	GTGGT <sup>U</sup> CGGCTGGACC	GACTGGAACCTTGCC	CTGAACCCCGAAGGA			1320
AsnLeuLeuTyrHis	ValValGlyTrpThr	AspTrpAsnLeuAla	LeuAsnProGluGly			
GGACCCAATTGGGTG	CGTAACTTTGTGCGAC	AGTCCCATCATTGTA	GACATCACCAAGGAC			1380
GlyProAsnTrpVal	ArgAsnPheValAsp	SerProIleIleVal	AspIleThrLysAsp			
ACGTTTTACAAACAG	CCCATGTTCTACCAC	CTTGCCATTTCAGC	AAGTTCATTCTGAG			1440
ThrPheTyrLysGln	ProMetPheTyrHis	LeuGlyHisPheSer	LysPheIleProGlu			

Mutations made to the Gcc insert are underlined

Figure 4B (continued on next page)

10	20	30	40	50	60	
123456789012345	678901234567890	1234567890	123456789012345	678901234567890		
GGCTCCAGAGAGTG	GGGCTGGTTGCCAGT	CAGAAGAACGACCTG	GACGCAGTGGCATTG			1500
GlySerGlnArgVal	GlyLeuValAlaSer	GlnLysAsnAspLeu	AspAlaValAlaLeu			
ATGCATCCCGATGGC	TCTGCTGTTGTGGTC	GTGCTAAACCGCTCC	TCTAAGGATGTGCCT			1560
MethHisProAspGly	SerAlaValValVal	ValLeuAsnArgSer	SerLysAspValPro			
CTTACCATCAAGGAT	CCTGCTGTGGGCTTC	CTGGAGACAATCTCA	CCTGGCTACTCCATT			1620
LeuThrIleLysAsp	ProAlaValGlyPhe	LeuGluThrIleSer	ProGlyTyrSerIle			
CACACCTACCTGTGG	CATCGCCAGTGATGG	AGCAGATACTCAAGG	AGGCACTGGGCTCAG			1680
HisThrTyrLeuTrp	HisArgGln					
				NotI		
CCTGGGCATTAAAGG	GACAGAGTCAGCGAA	TTCTGCAGATATCCA	TCACACTGGCGGCCG			1740
C						1741

Mutations made to the Gcc insert are underlined

Figure 4B (continued)

